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Investigating the Bioactive Constituents of the Edible Blue-Green Alga *Spirulina platensis*

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Date Submitted:
Abstract

More people die annually from cardiovascular disease than from any other cause. Two risk factors for cardiovascular disease are hyperlipidemia and inflammation. Current drugs that are prescribed to regulate lipid levels often have adverse effects such as liver dysfunction. Blue-green algae (BGA), also known as cyanobacteria, have been consumed for years for their health benefits because they are believed to increase energy and prevent disease. One genus of edible blue-green algae is *Spirulina plantesis* (SP, Spirulina). Various studies have shown that Spirulina may have anti-cancer and anti-inflammation and anti-bacterial properties. In a previous study, *Spirulina platensis* lowered triglyceride and cholesterol levels in hyperlipidemic rats. The rats were fed a Spirulina supplemented diet and their lipid levels were measured by their fecal output and intestinal absorption. However, the study did not involve isolation of the compounds responsible for this biological activity. The goal of this project is to isolate the specific compound(s) responsible for the anti-inflammatory and hypolipidemic effects of *Spirulina platensis*. 
Introduction

Cardiovascular disease is the number one cause of death in men and women in the United States (Ford 2012). Cardiovascular disease is defined as changes in the physiology and structure of the heart or blood vessels that impairs function (AHA 2015). This research project focused on two risk factors for cardiovascular disease: hyperlipidemia and inflammation. Inflammation is an immune system response to harmful stimuli. Hyperlipidemia is an increase in triglycerides in the blood plasma (Gotto 1977). Hypercholesterolemia is a subset of hyperlipidemia defined as an increase in total cholesterol levels in the blood (Gotto 1977).

Hypercholesterolemia can be familial or can be triggered by lifestyle and diet (Haines 2013). The two processes involved in cholesterol regulation are cholesterol synthesis and cholesterol absorption from the diet. Cholesterol synthesis begins in the mevalonate pathway (Figure 1). The rate-limiting enzyme in cholesterol biosynthesis is 3-hydroxy-3-methyl-glutaryl-CoA Reductase (HMG-CoA Reductase or HMGR), the enzyme that converts HMG-CoA into mevalonate (Haines 2013). Mevalonate is converted to squalene and then to lanosterol. There are two pathways that convert lanosterol to cholesterol: the Kandutch-Russell pathway and the Bloch pathway. The Kandutch-Russell pathway converts lanosterol to lathosterol and 7-dehydrocholesterol before converting it to cholesterol while desmosterol is the intermediate in the Bloch pathway (Wu 2014) (Figure 2). The intermediates of cholesterol synthesis are used as biomarkers to determine the rate of in vivo cholesterol synthesis.
Figure 1. Enzymes and products of the mevalonate pathway (Haines 2013).

Figure 2. Biosynthesis of cholesterol through the Bloch and Kandutsch-Russell Pathway (Wu 2014).
Dietary cholesterol absorption begins in the small intestine. Cholesterol particles form micelles and pass from the lumen of the intestine into the blood (Elshourbagy 2014). Cholesterol is insoluble in the plasma and is transported as lipoproteins. The types of lipoproteins in circulation are high-density lipoproteins (HDL), intermediate density lipoproteins (IDL) low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) (Elshourbagy 2014). The level of density of the lipoproteins represents the amount of protein they carry, with HDL composed of the highest level of protein (Elshourbagy 2014). HDL and LDL are the most commonly monitored lipoproteins. HDL and LDL are colloquially referred to as “good cholesterol” and “bad cholesterol” respectively. HDL facilitates reverse cholesterol transport, the process in which excess cholesterol from cells is returned to the liver to be degraded (Elshourbagy 2014). Dyslipidemia is a decrease in the amount of HDL. LDL transports cholesterol from the liver to peripheral tissues. A buildup of LDL in the plasma leads to inflammation and atherosclerosis (Elshourbagy 2014).

LDL buildup in blood vessels puts stress on the vessel walls (Salisbury 2014). This stress damages the vessel walls inducing the inflammatory response. Macrophages and other immune cells recruited as part of the inflammatory response secrete pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β, IL-1B) (Salisbury 2014). These cytokines further progress the inflammatory state. Animal studies have shown that HDL decreases inflammation by removing LDL from vessel walls (Elshourbagy 2014).

The lipid hypothesis states that high LDL levels correlate positively with cardiovascular disease risk and high HDL levels correlate negatively (Tobert 2003). In 1984, the National Institute of Health began to urge physicians to monitor the diets and nutrition of their patients with hypercholesterolemia in order to control their LDL levels. The first drugs employed to treat
hypercholesterolemia inhibited enzymes at the end stages of the mevalonate pathway. However, most of these drugs had adverse side effects such as the development of cataracts and toxicity. As a result, research shifted to the rate-limiting enzyme in cholesterol synthesis, HMGR. The first HMGR inhibitor was called compactin. It was isolated from a bacterial broth culture by a Japanese scientist in the 1970’s. The most common class of HMGR inhibitors is the statin family. Statins bind competitively to the enzyme’s active site. The first clinically approved statin, lovastatin, was isolated from a fermented *Aspergillus terreus* broth (Tobert 2003). Eight derivatives of lovastatin and compactin have been synthesized (Figure 5). Other treatments for hypercholesterolemia include fibrates, bile acid sequestrates, and nicotinic acid.

Current treatment of hyperlipidemia focuses on monitoring specific lipid levels and reducing all cardiovascular disease risk factors. Data derived from clinical trials and observational studies have led to the development of three health questions recommended by the American Heart Association to determine therapy options. Rather than basing therapies solely on LDL levels, treatment intensity is decided based on age, comorbidity, in addition to LDL levels (Pines 2014). The most common drug therapies for hypercholesterolemia are statins in combination with diet modification. Side effects of statin use include muscle and liver damage, impairment of cognitive function, and increased blood sugar (Dimmit 2015).

Lipid metabolism and inflammation are interconnected. Circulating cholesterol and lipids are known to stimulate the inflammatory response (van Diepen 2013). Pro-inflammatory cytokines also have a direct effect on lipid metabolism. As a result of this connection, lipid-lowering drugs can also have anti-inflammatory effects. Statins, niacin, and fibrates decrease inflammation by interacting directly with inflammatory pathways (van Diepen 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) are a major class of drugs used to treat pain and
inflammation. Aspirin is an NSAID that decreases inflammation by reducing the synthesis of prostaglandins. High-dose aspirin treatment also reduce lipid and cholesterol levels (van Diepen 2013). However, a study found that patients with cardiovascular disease who use the NSAIDs diclofenac and rofecoxib are five times as likely to suffer a myocardial infarction than patients with cardiovascular disease that don’t use those NSAIDs. (Schjerning 2014).

One mechanism of action to reduce inflammation is to target pro-inflammatory cytokines. In low doses, a drug called methotrexate, serves as an anti-inflammatory drug by block the pro-inflammatory cytokine interleukin-1beta from binding to its receptor (van Diepen 2013). Methotrexate has been shown to increase HDL levels in patients with rheumatoid arthritis (van Diepen 2013). Possible side effects from methotrexate use include gastrointestinal bleeding and edema. Tocilizumab, infliximab, and canakunimab also target pro-inflammatory cytokines. These drugs decrease the levels of the pro-inflammatory cytokines tumor necrosis factor alpha, interleukin 6 and interleukin-1beta respectively (van Diepen 2013). Specifically, these drugs disrupt cytokine signaling by degrading the cytokine receptors. Anti-cytokine drugs can have adverse gastrointestinal effects and interact with medicines such as birth control and blood thinners.

As a result of the side effect of common drug therapies, research on natural products with hypolipidemic properties is necessary. Historically, natural products have been the source of many drugs and drug therapies. Between the years 1981 and 2010, 34% of drugs based on small molecules came from natural product sources (Harvey 2015). Researchers have been analyzing techniques that have utilized natural products for years, such as traditional Chinese medicine, in an effort to find natural products that match their targets.
Figure 5. Structures of lovastatin and compactin drug derivatives (Tobert 2003).
Natural product extracts usually contain multiple compounds, which can be obstacle or an advantage. An array of compounds could mean that a single natural product could work on multiple targets. On the other hand, inactive compounds present in larger amounts may block the activity of less abundant active compounds. Complications with isolation and synthesizing of natural products have caused a decline in natural product drug discovery over the past two decades (Harvey 2015).

Table 1 shows 23 natural compounds and extracts that decreased or inhibited HMGR and had additional hypolipidemic effects. The compounds and extracts are divided into in vivo and in vitro studies. Compounds are further divided by the method of action: transcriptional or enzymatic regulation.

One of the particularly interesting compounds is naringenin, a flavonoid from grapefruit, exerts hypolipidemic effects in vitro. Rat hepatocytes treated with 200µM of naringenin had a 60% reduction in triglyceride production. Naringenin also increased LDLR expression and decreased HMGR and HMG-CoA synthase (HMGCS) expression (Goldwasser 2010). HMGCS is an enzyme involved in cholesterol biosynthesis. This compound has great medicinal potential because it has shown anti-inflammatory and hypolipidemic effects in vivo and in vitro. Additionally, the effect of naringenin on fatty acid oxidation and cholesterol biosynthesis indicates that it may play an important role in the regulation of lipid metabolism (Goldwasser 2010).
<table>
<thead>
<tr>
<th>Compound/Extract</th>
<th>Source</th>
<th>Method of Action</th>
<th>Dosage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amyloid beta</td>
<td>Amyloid precursor protein</td>
<td>Inhibits HMGR</td>
<td>n/a</td>
<td>Grimm 2007</td>
</tr>
<tr>
<td>Aglycone isoflavones</td>
<td>Korean soybean paste</td>
<td>Inhibits HMGR</td>
<td>30 µg/150µl</td>
<td>Sung 2010</td>
</tr>
<tr>
<td>Inhibitory peptide GPDPGG</td>
<td>Glycine soja</td>
<td>Binds competitively to HMGR</td>
<td>IC₅₀ 1.5µM</td>
<td>Pak 2008</td>
</tr>
<tr>
<td>Lipid extract</td>
<td>Nostoc commune</td>
<td>Inhibits SREBP-2 decreasing HMGR mRNA.</td>
<td>100mg/L</td>
<td>Rasmussen 2008</td>
</tr>
<tr>
<td>M2Columbamine</td>
<td>Rhizoma copitidis</td>
<td>Phosphorylates AMPK. AMPK inhibits HMGR in the liver</td>
<td>15pM</td>
<td>Cao 2011</td>
</tr>
<tr>
<td>Roxyloside</td>
<td>Rosa damascena</td>
<td>Inhibits HMGR</td>
<td>0.12 mg/mL</td>
<td>Kwon 2012</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algae powder</td>
<td>Schizochytrium</td>
<td>Decreased intestinal cholesterol absorption and HMGR mRNA</td>
<td>10g/kg bw</td>
<td>Wu 2003</td>
</tr>
<tr>
<td>Berberine</td>
<td>Coptis chinensis</td>
<td>Decreased hepatic cholesterol upregulated LDLR</td>
<td>200mg/kg bw</td>
<td>Chang 2002</td>
</tr>
<tr>
<td>Bergamot polyphenols</td>
<td>Citrus bergamia</td>
<td>Selective inhibition of HMGR</td>
<td>20 mg/day</td>
<td>Mollace 2011</td>
</tr>
<tr>
<td>β-amyrin acetate and β-amyrin palmitate</td>
<td>Wrightia somniosas leaves</td>
<td>Inhibits HMGR by binding to active site</td>
<td>10mg/kg bw</td>
<td>Maurya 2012</td>
</tr>
<tr>
<td>Diallyldisulfide analogs</td>
<td>Allium sativum</td>
<td>Decrease HMGR mRNA</td>
<td>20mg/kg bw</td>
<td>Rai 2004</td>
</tr>
<tr>
<td>Ethalonic extract</td>
<td>Symplocos racemosa Roxb bark</td>
<td>Inhibited liver HMGR</td>
<td>400mg/kg bw</td>
<td>Durkar 2014</td>
</tr>
<tr>
<td>Gingerol</td>
<td>Zingiber officinalis</td>
<td>Increased LDLR mRNA, decreased HMGR mRNA</td>
<td>400mg/kg bw</td>
<td>Namma 2010</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>Celastus paniculatus seed</td>
<td>Inhibits HMGR</td>
<td>65 µg/kg</td>
<td>Paul 2010</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>Aconitum heterophyllum</td>
<td>Inhibits HMGR</td>
<td>400mg/kg</td>
<td>Subash 2012</td>
</tr>
<tr>
<td>Monoterpenes geraniol</td>
<td>Lemongrass oil</td>
<td>Decreases translational efficacy of HMGR</td>
<td>100mg/kg bw</td>
<td>Jayachandran 2015</td>
</tr>
<tr>
<td>Naringenin</td>
<td>Grapefruit</td>
<td>Induces LDLR transcription, inhibits SREBP dependent HMGR</td>
<td>200µM</td>
<td>Goldwasser 2010</td>
</tr>
<tr>
<td>Sophoroside</td>
<td>Sophora japonica</td>
<td>Downregulates SREBP</td>
<td>10µM</td>
<td>Wu 2014</td>
</tr>
<tr>
<td>Sterol extracts</td>
<td>Schizochytrium sp</td>
<td>Upregulates LDLR down regulates HMGR</td>
<td>0.30 g/kg</td>
<td>Chen 2013</td>
</tr>
<tr>
<td>Red koi extract</td>
<td>Red koi</td>
<td>Inhibits HMGR</td>
<td>n/a</td>
<td>Wu 2003</td>
</tr>
<tr>
<td>Rutin</td>
<td>Plant-derived dietary flavanoid</td>
<td>Decreased HMGR mRNA</td>
<td>200 µM</td>
<td>Cheng 2011</td>
</tr>
<tr>
<td>RVS glycoprotein</td>
<td>Bius vernieghia Stokes</td>
<td>Inhibits HMGR</td>
<td>100mg/kg</td>
<td>Sun 2006</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>Plants</td>
<td>Decreased HMGR mRNA levels</td>
<td>0.02% w/w</td>
<td>Do 2011</td>
</tr>
</tbody>
</table>
Figure 6. Structure of naringenin (Goldwasser 2010).

Figure 7. Structures of active bergamot flavonoids (Mollace 2011).
Another citrus plant with bioactivity is bergamot (Figure 7). Bergamot is known for its high content and diversity of flavonoids. In a clinical study, polyphenol fractions of bergamot juice were freeze-dried and turned into 500mg tablets. These tablets were given to an experimental group of patients with hypercholesterolemia once a day. The control group of hypercholesterolemic patients was given a daily 500mg placebo pill. After 30 days, patients who took the bergamot pills had a 20% reduction in total cholesterol and LDL cholesterol (Mollace 2011). HPLC analysis of the bergamot fraction revealed five flavonoids that made up 26-28% of the active fraction (Mollace 2011)

*Spirulina platensis* (also referred to as SP and Spirulina) is a prokaryotic blue green alga also known as cyanobacteria. These organisms are photosynthetic but do not have plant cell walls, nuclear membranes, or internal organelles, resembling bacterial cells (Singh 2007). Spirulina was first isolated from a freshwater river in 1827 (Ciferri 1983). Indigenous populations in Africa and Mexico are known to have cultivated Spirulina from lakes and consumed the dried alga (Ciferri 1983). Spirulina has many bioactive components such as chlorophylls, carotenoids, amino acids, minerals, phycocyanin and secondary metabolites (Singh 2007) (Figure 8a). Spirulina is known as a superfood because of the abundance of nutrients it contains. The amounts of these various components depend on the environment in which the Spirulina is grown (Singh 2007). Cyanobacteria are known to have a diverse range of biological activity such as anti-malarial, anti-inflammatory, and anti-viral activity (Figure 8b).
Figure 8. A) Composition of *Spirulina platensis*. B) known biological activity of cyanobacteria (Singh 2007).
In a previous study done by Dr. Ji-Young Lee, the anti-inflammatory and hypolipidemic properties of two edible genera of blue-green algae have been shown (Yang 2011). An extract of the blue green algae *Nostoc commune* var. *sphaeroides* Kützing decreased the mRNA levels of pro-inflammatory cytokines, HMGR and the LDL receptor (LDLR) in RAW macrophages and HepG2 cells. In vivo studies of edible blue green algae with the C57BL/6J mice model of hyperlipidemia have had similar results. Mice fed a high fat diet supplemented with Nostoc and Spirulina for 4 weeks showed a change in the composition of intestinal bacteria and a decrease in plasma triglyceride and cholesterol levels (Rasmussen 2009). These hypolipidemic effects are attributed to an increased intestinal absorption of lipids and an increased fecal output (Ku 2013). The long-term effects of Spirulina and Nostoc were studied in vivo by supplementing the diets of mice with the BGA for six months. The mice from the experimental group showed no adverse affects in comparison to the control group.

Although these studies have demonstrated the anti-inflammatory and hypolipidemic properties of blue-green algae, the compounds responsible the bioactivity are unknown. The goal of my project was to isolate the compound(s) responsible for the effects of *Spirulina platensis*. Compound structures will be determined and these will be tested on biological assays.

**Experimental**

Fifty grams of dried Spirulina was obtained from Earthrise Nutritionals in Irvine, CA. The dried algae was weighed and incubated overnight in 300mL of a 2:1 ratio of dichloromethane (DCM) to methanol. After incubation, the alga and solvent were poured into a funnel with coarse filter paper to separate the solvent from the alga. The alga was incubated 8-10 times until an exhaustive extraction was performed, resulting in 6.43 g of crude extract. The
crude extract was rehydrated with hexanes, combined with silica (Pittsburg, PA) and passed through a flash column. Solvents (500mL each) were passed through the column with added air pressure according to the following ratio: A: 100% hexanes, B: 10% ethyl acetate (EtOAc)/hexanes, C: 20% EtOAc/hexanes, D: 40% EtOAc/hexanes, E: 60% EtOAc/hexanes, F: 80% EtOAc/hexanes, G: 100% EtOAc, H: 25% MeOH/EtOAc, I: 100% MeOH, J: 100% DCM and 100% MeOH.

GAPSP1 fractions A-J were analyzed using liquid chromatography mass spectroscopy (LCMS) with Agilent ESI single quadrupole mass spectrometer (Santa Clara, CA) paired to Agilent high performance liquid chromatography (HPLC) system with a G1311 quaternary pump, G1322 degasser, and a G1315 diode array detector using an Eclipse XDB-C18 (4.6 × 150 mm, 5 µm) RP-HPLC column. Fractions that had shown activity in the previous study were also tested on the Agilent HPLC system.

The bioactivity of SP was tested for HMG-CoA Reductase (HMGR) expression by treating a HepG2 cell with the GAPSP1 fractions dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 µg/mL of media. The HepG2 cells were incubated for 24 hours. Interleukin-1β (IL-1b) expression was tested using RAW 264.7 mice macrophage bioassay. The fractions were applied to at a concentration of 1 µg/mL. Before fractions were added, the macrophages were treated with 100 ng/mL of lipopolysaccharide (LPS) to stimulate inflammation.

After analysis of the LCMS chromatograms, GAPSP1C was selected for further fractionation by reverse phase solid phase extraction (SPE) on a DSC-18 500mg column using 60 mL of the following solvent systems: 1: 25% MeOH/water (H₂O) 2: 50% MeOH/H₂O 3: 75% MeOH/H₂O 4: 100% MeOH 5: 100% EtOAc.
GAPSP1C fractions 1-5 were analyzed using LCMS. GAPSP1C4 was analyzed on HPLC using 75% ACN and 70% ACN. GAPSP1C4A, GAPSP1C4B, GAPSP1C4W, GAPSP1C4A.E were collected from GAPSP1C4 using HPLC at 60% ACN over 30 minutes (Figure 5). GAPSP1C4B was 60% pure and 0.84 mg of the compound was isolated (Figure 6). GAPSP1C and GAPSP1C4B proton NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer (500.13 MHz 1 H, 125.65 MHz 13C).

Results

In our previous study, the D fraction (40% EtOAc/hexanes) eluted during the normal phase flash column chromatography of the initial crude SP extract, reduced IL-1B and HMGR expression (Figure 9). Further HPLC isolation of the D fraction yielded compounds, CSKSP1D1+2. CSKSP1D1+2 was isolated at 40% ACN and eluted at 8.5-9.5 minutes. The isolated compounds also had a repressive effect on HMGR and IL-1B (Figure 10).

A similar peak to those found in the original compound was found in the C fraction. GAPSP1C (Figure 15) eluted at 20% EtOAc/hexanes during normal phase flash column chromatography of the crude SP extract. SPE of GAPSP1C yielded five fractions. Compound 1 and 2 were most prevalent in GAPSP1C4 but were also present in fractions GAPSP1C3, and GAPSP1C5. HPLC isolation at 60% ACN for 30 minutes yielded GAPSP1C4A eluted at 17 minutes, GAPSP1C4B eluted at 26 minutes, GAPSP1C4A.E, and GAPSP1C4W (Figure 16). GAPSP1C4B is 60% pure and contains a similar peak to that found in CSKSP1D1+2 (Figure 17). GAPSP1 fractions A, B, H, I, J repressed IL-1b expression in RAW 264.7 macrophages (Figure 14). The fractions did not show significant activity against HMGR expression.
Figure 9. Bioactivity of CSKSP fractions in A) HepG2 cells and B) RAW 264.7 macrophages (Ku 2014).
Figure 10. Bioactivity of CSKSPD fraction and CSKSPD1+2 in A) HepG2 cells and B) RAW 264.7 macrophages (Ku 2014).
Figure 11. A) HPLC chromatogram and B) mass trace of CSKPD1+2.
Figure 12. Proton NMR spectrum of CSKSPD1+2.
Figure 13. GAPSP1 fraction tree.

Figure 14. IL-1b expression in RAW 264.7 macrophages after treatment with GAPSP1 fractions.
Figure 15. A) HPLC chromatogram and B) mass trace of GAPSP1C.
Figure 16. HPLC chromatogram of GAPSP1C4 at 60%ACN over 30 minutes. GAPSP1C4A, GAPSP1C4B, GAPSP1C4A.E, GAPSP1C4W are highlighted.
Figure 17. A) HPLC chromatogram and B) mass trace of GAPSP1C4B.
**Figure 18.** Proton NMR of GAPSP1C4
Discussion

Re-isolation of CSKSPD1+2 proved to be challenging because there was only a very small amount of the compounds isolated. This limited the number of analyses that could be performed. Also, large scale isolation of this compound was difficult because the active compound was a very small portion of the crude Spirulina extract. Further analysis will be done to determine if both compound 1 and 2 are active and if they act synergistically.

The original mass that was sought after in the GAPSP1 fractions was 369.1. This was the mass of the most prevalent peak in the initial SP D fraction. Conversely, the isolated bioactive compound, CSKSPD1+2, does not contain a peak with that mass. Future studies will pursue the masses present in CSKSPD1+2.

GAPSP1C fraction was not active in the HMGR or IL-1B bioassays. This may be a result of more abundant nuisance compounds overshadowing the anti-inflammatory or HMGR-repressing compounds that are present in smaller amounts. GAPSP1C4B will be tested in the HMGR and IL-1B bioassays to analyze the bioactivity. However, because only 0.48 mg were isolated, more of the compound will be necessary for structure determination.

CSKSPD1+2 activity against HMGR and IL-1B mRNA demonstrates that the compounds are active transcriptionally. Future studies should analyze if the compounds also inhibit the enzymatic activity of HMGR and/or prevent IL-1B from binding to its receptor.

Conclusion and Future Directions

The goal of this study was to isolate the compounds responsible for the hypolipidemic and anti-inflammatory effects of the edible blue-green alga, *Spirulina platensis*. Initial
fractionation of a crude Spirulina extract resulted in a bioactive D fraction which was further fractionated into the bioactive compounds CSKSPD1+2. A second fractionation of a crude Spirulina extract yielded a fraction, GAPSP1C that contained similar peaks to the original active D fraction. The peak of interest was isolated in GAPSP1C4B, but not enough of the compound was isolated to determine the chemical structure. Future studies will involve re-isolation of the compound and comparison of the bioactivity of GAPSP1C4B to CSKSPD1+2. Additionally, the synergistic activity of these two compounds and the other bioactive fractions will be analyzed.
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